Electronmicroscopy of Sciatic Nerves in Aging Rats with Spontaneous Radiculoneuropathy

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ABSTRACT. The sciatic nerves of fourteen 104- to 135-week-old rats with spontaneous radiculoneuropathy were examined by light and electron microscopy. The most conspicuous changes were the nerve fiber degeneration indicative of wallerian degeneration and regeneration. Along with these changes, shrinking axons with disproportionately thicker myelin sheaths could sometimes be observed in large myelinated nerve fibers. In these myelinated fibers, there were also demyelinating changes characterized by wide distention of myelin sheaths with shrinkage of axons which are the most prominent changes in the spinal nerve roots in the radiculoneuropathy. Among the nerve fibers showing these demyelinating changes, axonal degradation following shrinkage of axons could be detected in several fibers. Our findings may suggest that shrinking axons in the spinal nerve roots cause axonal degradation and subsequent destruction of nerve fibers in the more distal portion of the peripheral nervous system in aging rats with radiculoneuropathy. — KEY WORDS: aging rat, radiculoneuropathy, sciatic nerve, ultrastructure.

Radiculoneuropathy is a spontaneous degenerative disease in the peripheral nervous system, which might be a cause of posterior paralysis found in aged rats more than 2 years old [1–4, 7, 9–11, 16]. In the proximal portion of affected peripheral nerves, demyelinating changes characterized by blebbing of myelin sheaths in myelinated nerve fibers with shrinkage of axons have been found. This radicular lesion was suggested to occur secondary to shrinkage of axons [10]. On the contrary, wallerian-type degeneration and loss of myelinated nerve fibers have been seen in the distal peripheral nerves such as plantar and tibial nerves in addition to the demyelination which might be caused by the pressure on these nerves [1, 4, 10, 16]. Krinke [10] surmised the cause of these lesions in the distal peripheral nerves that severely constricted axons in the spinal nerve roots might cause secondary degeneration of nerve fibers in the distal nerves. However, there is no morphological evidence to support that atrophic axons with myelin splitting are responsible for the destruction of nerve fibers in the distal nerves, although age-related changes in the distal nerves have been reported in older rats [5, 8, 14, 15].

We previously reported the ultrastructural changes in the spinal nerve roots and dorsal root ganglia of aged rats with spontaneous radiculoneuropathy [11]. In order to investigate the relationship of the lesions between the proximal and distal portions in the peripheral nerves of these rats, the present study deals with the ultrastructural changes in the intermediated portion of the peripheral nerves, the sciatic nerve, which was obtained from the animals used in the previous study [11].

MATERIALS AND METHODS

Seven male and seven female Sprague-Dawley SPF rats more than 104 weeks old (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka) which were reared as untreated controls in a
30-month chronic toxicity test were examined in this study. They are a subset of the 12 males and 14 females examined in a previous study [11]. Of these 14 rats, 6 males and 5 females became moribund and the remaining animals (1 male and 2 females) were subjected to terminal kill at 135 weeks old (Table 1).

Among 11 moribund rats, 3 animals of either sex, including 1 male and 2 females with posterior paresis, were anesthetized with chloral hydrate injected intraperitoneally. They were perfused through the left cardiac ventricle with physiologic saline and then with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The sciatic nerves were sampled and fixed for additional three hours in the same fixative. The remaining rats (four animals of each sex), including 1 male and 2 females with the neurological signs, were killed by an overdose of ether. The same nerve tissues were removed immediately, and immersed in phosphate-buffered 3% glutaraldehyde for 6 to 12 hours. Both perfused and non-perfused nerve tissues were post-fixed with 1% osmium tetroxide for two hours, dehydrated in graded alcohols, cleared in n-butyl glycidyl ether and embedded in epon. Semi-thin sections, 1 μm thick, were cut both transversely and longitudinally, and stained with toluidine blue-basic fuchsin in borax for light microscopy. Ultrathin sec-

Table 1. Number of animals examined ultrastructurally

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<td>With posterior paresis</td>
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<td>Moribund kill</td>
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<td>104 to 112 weeks old</td>
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<td>129 to 134 weeks old</td>
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<td>Terminal kill</td>
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<td>135 weeks old</td>
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<td>2</td>
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a) Animal subjected to perfusion fixation.
RESULTS

_Light Microscopy:_ In the animals showing neurological signs, breakdown of myelin sheaths into irregular-shaped masses or ovoids similar to those observed in wallerian degeneration as well as loss of nerve fibers were the most conspicuous changes (Fig. 1). The longitudinal sections revealed that these fibers had fragmented axons and separation into a discontinuous series of myelin ovoids. Blebbing or wide distention of myelin sheaths was also seen in myelinated nerve fibers with large diameter (Figs. 1 and 2) accompanied by invasion of myelin debris laden macrophages into the intramyelinic spaces. In the demyelinated nerve fibers, the axon showed a reduction in diameter and complete destruc-

Fig. 2. Sciatic nerve from a rat with neurological signs. Degenerated nerve fibers showing complete destruction (C) and demyelinated nerves with distention of myelin and invasion of macrophage (M) in intramyelinic spaces. Axons (A) appear normal. Some axons are invested by thin myelin sheaths (arrow heads). 1 μm section, toluidine blue-basic fuchsin. ×1,520.

Fig. 3. Sciatic nerve from a rat without neurological signs. Regenerated nerve fibers showing bundles of 2 to 5 small myelinating fibers (arrow heads). 1 μm section, toluidine blue-basic fuchsin. ×1,600.

Fig. 4. Nerve fiber degeneration similar to wallerian degeneration. Axonal degradation with intact myelin sheaths (A) and breakdown of myelin into concentric myelin ovoid with collapse of axon (B). ×2,100.
Fig. 5. Shrinking axons (arrows) with disproportionally thicker myelin sheaths. ×5,650.

Fig. 6. Degradation of neurofilaments showing fine granular dark profile (arrow) in nerve fiber with shrinkage of axon and distention of myelin sheaths. ×5,650.

Fig. 7. Breakdown of distended myelin sheaths following destruction of atrophic axon. ×7,800.
tion of the sheaths was also seen (Fig. 2). There were increases of collagen fibers and denervated Schwann cell columns in areas disclosing loss of nerve fibers. Thiny myelinated nerve fibers and bundles of 2 to 5 small myelinating fibers were also commonly found in these lesions (Figs. 1–3). Similar changes of nerve fibers, though less severe, were also observed in animals without neurological signs.

Electron Microscopy: In the sciatic nerves of all the rats examined, the following wallerian-type degeneration were most commonly seen in myelinated nerve fibers of various diameters but not in unmyelinated fibers; accumulations of intra-axonal organelles, degradation of neurofilaments in axons showing fine granular dark profile with intact myelin sheaths, disoriented layerings or infoldings of myelin sheaths with axonal destruction and breakdown of myelin sheaths into concentric membranous structures with collapse of the axons. These changes were more conspicuous in the animals showing neurological signs and more with advanced age (Fig 4). Together with these destructive changes, shrinking axons with disproportionately thicker myelin sheaths could be sometimes detected in large myelinated fibers (Fig. 5). The density of neurofilaments and microtubules increased strikingly within these axis cylinders. Demyelinating changes characterized by wide distention of myelin sheaths were sometimes observed in large myelinated fibers. Most of the axons with these demyelinating changes showed marked reduction in diameter. Among these, however, several fibers disclosed axonal destruction such as degradation of neurofilaments with fine granular dark profile (Fig. 6). Furthermore, secondary destruction of nerve fibers following these axonal degradations and demyelinating changes could be detected (Fig. 7). In the area showing complete destruction of fibers, there was also invasion of macrophages.

The changes known as age-related abnormalities [8, 15] were frequently observed in nerve fibers of otherwise normal appearance in all the animals.

Reparative changes of nerve fibers were more frequent in rats with neurological signs. Fibers invested by disproportionately thin myelin sheaths as compared to the axon diameter
was observed (Fig. 8). There was also denuded axons enclosed by proliferating Schwann cells and axons invested by new myelin sheaths consisting of several major dense lines within a profusely folded basement membrane. These axons showed increased density of neurofilaments and microtubules. Some supernumerary Schwann cell processes were present around remyelinated fibers. The bands of Büngner with multiple axonal sprouts within a folded basement membrane were commonly found in addition to the remyelination (Fig. 9). There was also bundles of three to five regenerating myelinating and unmyelinated fibers surrounded by a common basement membrane which indicate further progression of regeneration (Fig. 10). In the areas showing loss of nerve fibers, collagen fibers were prominent, and empty folds of basement membrane and Schwann cell collagen pockets (Fig. 11) were occasionally noticed.

**DISCUSSION**

In the present study, wallerian degeneration of various types [12] was frequently detected in the sciatic nerve. Shrinkage of axons and subsequent axonal degradation could be seen in myelinated fibers showing wide distention of myelin sheaths which is a significant morphological feature of the radioculoneuropathy in the spinal nerve roots of aging rats [9, 11]. This evidence in our study suggests that atrophic axons with wide distention of myelin sheaths cause axonal degradation and subsequent fiber destruction.

Age-related changes in the distal peripheral nerves have been already described in rats [5, 8, 14, 15]. In the plantar and tibial nerves, axonal degeneration and regeneration as well as segmental demyelination and remyelination occur frequently in rats more than 24 months old. Among these changes, demyelination which is different from that in the
spinal nerve roots and remyelination have been detected in the plantar nerve in the animals as early as 6 months old [14, 15]. This demyelination is considered to be due to pressure effects, since it is known that a pressure neuropathy consisting of demyelination is produced in the plantar nerves of guinea pigs kept in cages with wire mesh floors [6]. On the other hand, axonal degeneration and regeneration do not become evident in the plantar nerve until 15 months and in the tibial nerve until 18 months [14]. No definite decision has been made as to the occurrence of the axonal degeneration, although such a change is speculated to be attributable to a dying-back process [16] or possible neuronal loss [14]. However, attenuated axons, the early change of radiculoneuropathy, have been described to occur in the ventral lumbar roots of rats 13 and 14 months old [10]. In this report, it was surmised that severely constricted axons in the spinal nerve roots of the radiculoneuropathy might cause secondary degeneration of nerve fibers in the distal nerves. The time when the attenuated axons were first seen in the spinal nerve roots is approximately equal to that of axonal degeneration in the plantar nerve. The results of these studies and our study strongly support the hypothesis that axonal degeneration in the distal peripheral nerves of rats more than 15 months old is the secondary phenomenon following the attenuation of axons in the spinal nerve roots of the radiculoneuropathy.

Regenerative changes of myelinated nerve fibers which were common findings in the sciatic nerve are essentially the same as those observed in the reparative stage following nerve amputation or crush injury [13]. In addition, Schwann cell collagen pockets suggestive of neuronal loss were not observed frequently in this nerve. This evidence may imply that atrophic axons detected in the sciatic nerve have active capability to regenerate even though they have passed into complete axonal death. Furthermore, it is considered that definite damage so as to inhibit the regeneration is not induced in the cells of origin of these myelinated nerves, the ventral horn neurons and dorsal root ganglionic cells. This speculation might be supported by the previous evidence that no marked degenerative changes could be seen in these neuronal cells, except for increased incidences of lipofuscin granules [10, 11]. Remyelination was also a common finding in the sciatic nerve of aged rats. The remyelination observed was similar to that seen in the spinal nerve roots in our previous study [11] and may suggest that nerve fibers with demyelination due to atrophy of the axon did not always reveal complete destruction of nerve fibers.

In our previous study [11], the change in the spinal nerve roots was considered to be essentially a primary demyelination in aged rats with spontaneous radiculoneuropathy, since we could not notice any axonal degeneration following the shrinkage of axons. However, the present results may suggest that the morphological feature of this radiculoneuropathy is characterized by demyelination secondary to shrinkage of axons in the spinal nerve roots. The cause of this radiculoneuropathy in senile rats remains to be elucidated by further investigations.

REFERENCES


要　約

老齢ラットの自然発生性神経根神経症における坐骨神経の電子顕微鏡的観察：三森国敏・真坂敬三・中島信明・白須泰彦（残留農業研究所）——自然発生性神経根神経症を伴った40から135週齢ラットの坐骨神経を光学的および電子顕微鏡的に観察した。最も顕著な変化は、ワーラー変性を示唆する神経線維変性と軸索再生であった。これらの変化とともに、軸索の口径に比し不均衡な厚さの詰詰が続く詰詰性の軸索が大型の有髄神経線維に著しく観察された。これらの有髄線維においては、本神経症の神経根神経症において最も顕著な変化である軸索の詰詰を伴う軸索の著しい拡張を特徴とする脱髓鞘変性も認められた。これらの脱髓鞘変性を示す神経線維の中では、軸索の詰詰に続く軸索の変性を示す神経線維が散見された。以上のことから、神経根神経症を伴う老齢ラットの末梢神経遠位部における軸索変性およびそれに続く神経線維の崩壊は、近位部での軸索変性に起因する変化であることが示唆された。